IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND INTERFERENCES

Application of:	Pittenger, et al.
Serial No.:	09/319,521
Filed:	June 4, 1999
For:	Improved Chondrogenic Differentiation of Human Mesenchymal Stem Cells
Group:	1644
Examiner:	Belyavskyi

Commissioner for Patents
Box 1450
Alexandria, VA 22313-1450

BRIEF BEFORE THE BOARD OF APPEALS AND INTERFERENCES

SIR:

This is an appeal from the Final Rejection dated June 29, 2005.

REAL PARTY IN INTEREST

The real party in interest is Osiris Therapeutics, Inc., the assignee of the claimed subject matter of the above-identified application.

RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences with respect to the above-identified application.

STATUS OF CLAIMS

Claims 60-99 are pending, stand finally rejected, and are before the Board on Appeal. These claims are listed in the Appendix attached hereto.

Claims 1-59 have been cancelled without prejudice.

STATUS OF AMENDMENTS

No amendments after the Final Rejection have been filed.

SUMMARY OF CLAIMED SUBJECT MATTER

The present invention, in one aspect, is directed to a process for producing chondrocytes, as defined broadly in Claim 60, and to a process for inducing chondrogenesis in mesenchymal stem cells, as defined broadly in Claim 70. Such processes are effected by culturing mesenchymal stem cells in a chemically defined serum-free medium *in vitro* wherein the mesenchymal stem cells are associated in a three-dimensional format. The chemically defined serum-free medium comprises (1) a chemically defined minimum essential medium; (2) ascorbate or an analog thereof; (3) an iron source; (4) insulin or an insulin-like growth factor; (5) at least one chondroinductive agent or factor; and (6) a simple sugar, wherein the simple sugar is present in the medium in an amount of from about 3g/l to about 7g/l.

Applicants have discovered, as shown in the examples, that by culturing mesenchymal stem cells in a chondrogenic medium which includes a simple sugar at a concentration of from about 3g/l to about 7g/l, one obtains improved differentiation of mesenchymal stem cells into chondrocytes, as opposed to media which have a lower sugar concentration, such as, for example, media which have a glucose concentration which is the standard concentration present in "low glucose DMEM" (1g/l).

In another aspect of the present invention, as defined broadly in Claims 80 and 90, the mesenchymal stem cells are cultured in a chemically defined serum-free medium as hereinabove described, wherein the at least one chondroinductive agent or factor comprises TGF-β3.

Applicants also have discovered that TGF-β3 is a more effective chondroinductive agent than those used previously, such as dexamethasone, BMP-2, BMP-4, TGF-β1, inhibin A, chondrogenic stimulating activity factor, collagen Type I, or retinoic acid. For example, in Example 3, TGF-β3 was found to have an improved effect on chondrogenic differentiation of human mesenchymal stem cells *in vitro* when compared to TGF-β1.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following grounds of rejection are to be reviewed on appeal:

(i) the rejection of Claims 60-79 under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 5,908,784 (Johnstone), as evidenced by the Cellgro catalog and the U.S. Biological Catalogue (2004) and Williams, et al.; and

(ii) the rejection of Claims 80-99 under 35 U.S.C. 103 as being unpatentable over U.S. Patent No. 5,908,784 (Johnstone) as evidenced by the Cellgro catalog and the U.S. Biological Catalogue (2004) and Williams, et al. in view of U.S. Patent No. 5,368,858 (Hunziker).

ARGUMENT

The Rejection Under 35 U.S.C. 102(e)

With respect to the rejection under 35 U.S.C. 102(e), the U.S. Biological Catalogue was published in 2004, and Williams was published in 2003, and the Cellgro catalog was published on October 6, 2001. The above-identified application was filed on June 4, 1999, which is a Section 371 application of PCT Application No. PCT/US97/22022, filed December 5, 1997,

which claims priority based on provisional application Serial No. 60/035,274, filed December 6, 1996.

The Examiner relies upon Ex parte Erlich, 22 U.S.P.Q.2d 1463 (Bd. App. Int. 1992), to justify his reliance on the Cellgro catalog, the U.S. Biological Catalog (2004), and Williams as evidential references in that the Examiner states that, in accordance with Erlich, these references show the level of ordinary skill in the art at or around the time the invention was made, even though such references postdate the claimed invention.

Erlich holds, however, that in order for a reference which was published after the filing date of an application to be relied upon to show the level of ordinary skill of the art at the time of the claimed invention, it must state clearly what the level of ordinary skill in the art was at the time the invention was made. In Erlich, the Applicant established a date of completion of his invention of June 12, 1980. The Board held that a reference, published in July 1981, could be relied upon in a rejection under 35 U.S.C. 103 because the reference cited other references dated 1979 and 1980 which showed the level of ordinary skill in the art at that time. (Erlich, at 1464-1465.)

The U.S. Biological Catalog (2004) and the Cellgro catalog provide no information as to the level of ordinary skill in the art, with respect to producing chondrocytes from a culture of mesenchymal stem cells, as of June 4, 1999, the filing date of the above-identified application, or as of the December 5, 1997 filing date of Applicants' PCT Application No. PCT/US97/22022, or as of the December 6, 1996 filing date of Applicants' provisional application Serial No. 60/035,274. Therefore, pursuant to Erlich, the U.S. Biological Catalog (2004) and the Cellgro catalog are not prior art against the above-identified application, and therefore it is proper to address the rejection under 35 U.S.C. 102(e) with respect only to (i) Johnstone and (ii) Williams, to the extent that Williams allegedly refers to the level of ordinary skill in the art at the time the above-identified application was filed.

The Federal Circuit has held that anticipation is established only if all elements of an invention, as stated in a patent claim, are identically set forth in a single prior art reference. All

of the limitations must be disclosed by the reference either expressly or inherently. (*See Mehl/Biophile International Corp. v. Milgraum*, 192 F.3d 1362 (Fed. Cir. 1999) at 1365; 52 U.S.P.Q.2d 1303, at 1306; *Oney v. Ratliff*, 182 F.3d 893 (Fed. Cir. 1999); 51 U.S.P.Q.2d 1697; *Finnigan Corp. v. U.S. International Trade Commission*, 180 F.3d 1354 (Fed. Cir. 1999), at 1367; 51 U.S.P.Q.2d 1001, at 1009; *General Electric Co. v. Nintendo Co., Ltd.*, 179 F.3d 1350 (Fed. Cir. 1999), at 1356, 50 U.S.P.Q.2d 1910, at 1915.) Anticipation is a question of fact. (*Rockwell International Corp. v. United States*, 147 F.3d 1358 (Fed. Cir. 1998), at 1363; 47 U.S.P.Q.2d 1027, at 1031.)

Johnstone does not disclose or even remotely suggest to one of ordinary skill in the art a chondrogenic medium that includes a simple sugar in an amount of from about 3g/l to about 7g/l. The only specific concentration of glucose disclosed by Johnstone is 1g/l, which is in a medium known as Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG). Although, at Column 4, lines 31 and 32, Johnstone lists "Dulbecco's Modified Eagle's Medium (DMEM)," as an example of a medium which may be used to promote chondrogenesis of mesenchymal stem cells, DMEM does not inherently have a simple sugar concentration of from 3g/l to 7g/l. In fact, Johnstone discloses, and Examiner even admits at Page 3, lines 1-4 of the Final Rejection, that there are examples of DMEM that have a glucose concentration of only 1g/l, including the DMEM used specifically by Johnstone. Therefore, all of the limitations of Claims 60-79 are <u>not</u> disclosed, either expressly or inherently, by Johnstone.

In fact, contrary to the requirements for anticipation under 35 U.S.C. 102, the Examiner relies on an <u>additional</u> reference, Williams, in order to formulate the rejection. Williams, at Page 680, column 1, refers to Rosen, et al., <u>J. Bone Miner. Res.</u>, Vol. 9, pg. 1759 (1994), Johnstone, et al., <u>Exp. Cell Res.</u>, Vol. 238, pg. 265 (1998), and Mackay, et al., <u>Tissue Eng.</u>, Vol. 4, pg. 415 (1998) to show that bone morphogenic protein 2, TGF-β_1 or TGF-β_3 can induce mesenchymal cells to differentiate into chondrocytes.

Firstly, the Johnstone and Mackay papers were published in 1998, after the filing dates of Applicants' provisional and PCT applications and, therefore, are not prior art.

The Rosen paper is directed to the use of bone morphogenic protein 2(BMP-2) for differentiating clonal limb bud cells into cartilage or bone. Nothing in Rosen discloses or even remotely suggests to one of ordinary skill in the art a medium for producing chondrocytes from mesenchymal stem cells which includes, in addition to a chondroinductive agent, a chemically defined minimum essential medium, ascorbate or an analog thereof, an iron source, insulin or an insulin-like growth factor, and a simple sugar present in an amount of from about 3 g/l to about 7 g/l.

Thus, even the combination of Johnstone and Williams does not disclose all of the elements of Applicants' Claims 60-79, either expressly or inherently. In addition, the combination of Johnstone and Williams does not even remotely suggest to one of ordinary skill in the art that a culture medium having a simple sugar concentration from 3g/l to 7g/l may be used as part of a culture medium for mesenchymal stem cells for enabling the mesenchymal stem cells to differentiate into chondrocytes. Therefore, Johnstone does not anticipate Applicants' processes as claimed, nor does Johnstone render Applicants' processes as claimed obvious to one of ordinary skill in the art.

In addition, assuming, solely for the sake of argument, that the Cellgro catalog and the U.S. Biological Catalog (2004) were applied properly by the Examiner, such references add nothing to Johnstone and the Rosen paper cited by Williams. All that the Cellgro catalog discloses are various formulations of DMEM, one of which contains 1g/l of glucose, and others which contain 4.5g/l of glucose. The Cellgro catalog does not provide any suggestion to one of ordinary skill in the art as to the types of cells which may be cultured in DMEM including 4.5g/l of glucose, or that DMEM including 4.5g/ml of glucose may be employed in a medium for culturing mesenchymal stem cells in order to enable the mesenchymal stem cells to differentiate into chondrocytes.

The U.S. Biological Catalogue (2004) merely describes a medium known as BGJb, Fitton-Jackson modification, which contains 10g/l of D-glucose. The medium permits calcification and growth of cartilaginous embryonic bone. Nothing in the U.S. Biological Catalogue suggests to one of ordinary skill in the art that such medium may be employed in a

medium for culturing mesenchymal stem cells in order to enable the mesenchymal stem cells to differentiate into chondrocytes.

Therefore, if the Cellgro catalog and the U.S. Biological Catalogue were relied upon properly by the Examiner as evidentiary references, such references could not be relied upon as a basis for asserting a proper rejection for anticipation by Johnstone under 35 U.S.C. 102(e).

It is therefore respectfully requested that the rejection under 35 U.S.C. 102(e) be reversed.

The Rejection Under 35 U.S.C. 103

As noted hereinabove, the U.S. Biological Catalog and the Cellgro catalog are not effective prior art references. Therefore, the rejection under 35 U.S.C. 103 will be addressed only with respect to Johnstone, Hunziker, and the Rosen paper cited in Williams.

The differences between Johnstone and the Rosen paper cited in Williams and Applicants' claimed processes have been noted hereinabove. Johnstone and the Rosen paper cited in Williams clearly do not disclose or even remotely suggest Applicants' claimed processes to one of ordinary skill in the art.

Furthermore, with respect to Johnstone, as noted hereinabove, the only specific teaching in Johnstone is with respect to the concentration of glucose in a chondrogenic medium for culturing mesenchymal stem cells is to use a concentration of 1g/l of glucose in such a medium. Therefore, Johnstone teaches away from the present invention, and such teaching away from the invention is indicative of non-obviousness. (See W.L. Gore & Associates, Inc. v. Garlock., Inc., 220 U.S.P.Q. 303 (C.A.F.C. 1983), at 312; United States v. Adams, 383 U.S. 39 (1966).)

Also, Johnston discloses as chondroinductive agents glucocorticoids such as dexamethasone, a bone morphogenic protein, such as BMP-2 or BMP-4, TGF-ß1, inhibinA, or chondrogenic stimulating activity factor. The examples employ dexamethasone, TGF-ß1, and BMP-2, respectively, as the chondroinductive agents. Johnstone does not disclose or even

remotely suggest to one of ordinary skill in the art that TGF-ß3 may be used as a chondronductive agent, or that TGF-ß3, when employed in a chondrogenic medium, provides for improved differentiation of mesenchymal stem cells into chondrocytes. Therefore, Johnstone does not render obvious to one of ordinary skill in the art the embodiments of the invention defined by Claims 80-99.

The Examiner relies on Hunziker to show that TGF-β3 may be used in a composition for transforming repair cells into chondrocytes. Hunziker discloses the treatment and repair of defects or lesions in cartilage by filling the defect or lesion with a biodegradable matrix containing a proliferation agent, a transforming agent, and repair cells. TGF-β, including TGF-β3, may be used as a proliferation agent and/or as a transforming factor. Insulin-like growth factor also may be used as a proliferation agent.

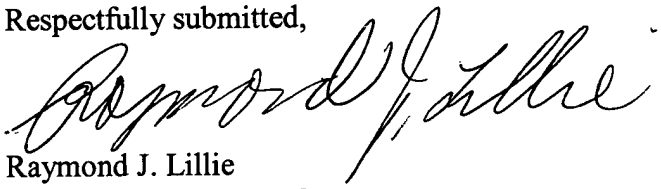
The composition of Hunziker, however, does not include a chemically defined minimal essential medium, ascorbate or an analog thereof, an iron source, and a simple sugar present in an amount of from 3g/l to 7g/l. Hunziker also does not even remotely suggest to one of ordinary skill in the art to include such components. Hunziker, therefore, does not disclose or even remotely suggest to one of ordinary skill in the art Applicants' processes as claimed. Thus, Hunziker does not render Applicants' processes as claimed obvious to one of ordinary skill in the art.

The combination of Johnstone, Hunziker, and the Rosen paper cited in Williams does not disclose or even remotely suggest to one of ordinary skill in the art a process for producing mesenchymal stem cells wherein the mesenchymal stem cells are cultured in a medium which includes all of the components claimed by Applicants, including a simple sugar which is present in the medium in an amount of from 3g/l to 7g/l. Applicants and only Applicants have discovered that by culturing mesenchymal stem cells in a chondrogenic medium which includes a simple sugar at a concentration of from about 3g/l to about 7g/l, one obtains improved differentiation of mesenchymal stem cells into chondrocytes as opposed to media which have a lower sugar concentration, such as, for example, media which have a glucose concentration which is the standard concentration present in "low glucose DMEM" (1g/l). At best, the

combination of Johnstone, Hunziker, and Williams would suggest to one of ordinary skill in the art to supply a chondrogenic medium which includes glucose at a concentration of only 1g/l. Johnstone, Hunziker, and the Rosen paper cited in Williams, therefore, did not contemplate Applicants' improvement for producing chondrocytes from mesenchymal stem cells wherein there is included in the chondrogenic medium a simple sugar which is present in the medium in an amount of from about 3g/l to about 7g/l. Therefore, the combination of Johnstone, Hunziker, and the Rosen paper cited in Williams does not render Applicants' process as claimed obvious to one of ordinary skill in the art, and it is therefore respectfully requested that the rejection under 35 U.S.C. 103 be reversed.

For the above reasons and others, this application is in condition for allowance, and it is therefore respectfully requested that the rejections be reversed.

Respectfully submitted,



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APPENDIX - CLAIMS ON APPEAL

60. A process for producing chondrocytes from mesenchymal stem cells by culturing mesenchymal stem cells in a chemically defined serum-free medium in vitro wherein the mesenchymal stem cells are associated in a three-dimensional format, and wherein said chemically defined serum-free medium comprises (1) a chemically defined minimum essential medium; (2) ascorbate or an analog thereof; (3) an iron source; (4) insulin or an insulin-like growth factor; (5) at least one chondroinductive agent or factor; and (6) a simple sugar, said simple sugar being present in said medium in an amount of from about 3g/l to about 7g/l.

61. The process of Claim 60 wherein said mesenchymal stem cells are isolated, culture expanded human mesenchymal stem cells.

62. The process of Claim 60 wherein the mesenchymal stem cells are condensed into close proximity.

63. The process of Claim 62 wherein the mesenchymal stem cells are present as packed cells or a centrifugal cell pellet.

64. The process of Claim 60 wherein said at least one chondroinductive agent or factor is selected from the group consisting of (i) a glucocorticoid; (ii) a member of the transforming growth factor-ß super-family; (iii) a component of the collagenous extracellular matrix; and (iv) a vitamin A analog.

65. The process of Claim 60 wherein said at least one chondroinductive agent is a combination of dexamethasone and TGF-ß1.

66. The method of Claim 60, and further comprising placing said cells in a rigid porous vessel.

67. The method of Claim 66 wherein said rigid porous vessel is a ceramic cube.

68. The method of Claim 60 wherein said simple sugar is glucose.

69. The process of Claim 68 wherein said glucose is present in said serum-free medium in an amount of about 4.5 grams per liter.

70. A process for inducing chondrogenesis in mesenchymal stem cells by culturing mesenchymal stem cells in a chemically defined serum-free medium in vitro wherein the mesenchymal stem cells are associated in a three-dimensional format, and wherein said chemically defined serum-free medium comprises (1) a chemically defined minimum essential medium; (2) ascorbate or an analog thereof; (3) an iron source; (4) insulin or an insulin-like growth factor; (5) at least one chondroinductive agent or factor; and (6) a simple sugar, said simple sugar being present in said medium in an amount of from about 3g/l to about 7g/l.

71. The process of Claim 70 wherein said mesenchymal stem cells are isolated, culture expanded human mesenchymal stem cells.

72. The process of Claim 70 wherein the mesenchymal stem cells are condensed into close proximity.

73. The process of Claim 72 wherein the mesenchymal stem cells are present as packed cells or a centrifugal cell pellet.

74. The process of Claim 70 wherein said at least one chondroinductive agent is selected from the group consisting of (i) a glucocorticoid; (ii) a member of the transforming growth factor-ß super-family; (iii) a component of the collagenous extracellular matrix; and (iv) a vitamin A analog.

75. The process of Claim 70 wherein said at least one chondroinductive agent is a combination of dexamethasone and TGF-ß1.

76. The method of Claim 70, and further comprising placing said cells in a rigid porous vessel.

77. The method of Claim 76 wherein said rigid porous vessel is a ceramic cube.

78. The method of Claim 70 wherein said simple sugar is glucose.

79. The process of Claim 78 wherein said glucose is present in said serum-free medium in an amount of about 4.5 grams per liter.

80. A process for producing chondrocytes from mesenchymal stem cells by culturing mesenchymal stem cells in a chemically defined serum-free medium *in vitro* wherein the mesenchymal stem cells are associated in a three-dimensional format, and wherein said chemically defined serum-free medium comprises (1) a chemically defined minimum essential medium; (2) ascorbate or an analog thereof; (3) an iron source; (4) insulin or an insulin-like growth factor; (5) at least one chondroinductive agent or factor, wherein said at least one chondroinductive agent or factor comprises TGF-β3; and (6) a simple sugar, said simple sugar being present in an amount of from about 3g/l to about 7g/l.

81. The process of Claim 80 wherein said TGF-β3 is present in said medium in an amount of at least 5 ng/ml.

82. The process of Claim 81 wherein said TGF-β3 is present in said medium in an amount of from 5 ng/ml to 15 ng/ml.

83. The process of Claim 80 wherein said mesenchymal stem cells are isolated, culture-expanded human mesenchymal stem cells.

84. The process of Claim 80 wherein the mesenchymal stem cells are condensed into close proximity.

85. The process of Claim 84 wherein the mesenchymal stem cells are present as packed cells or a centrifugal cell pellet.

86. The process of Claim 80, and further comprising placing said cells in a rigid porous vessel.

87. The process of Claim 86 wherein said rigid porous vessel is a ceramic cube.

88. The process of Claim 80 wherein said simple sugar is glucose.

89. The process of Claim 88 wherein said glucose is present in said serum-free medium in an amount of about 4.5 grams per liter.

90. A process of inducing chondrogenesis in mesenchymal stem cells by culturing mesenchymal stem cells in a chemically defined serum-free medium *in vitro* wherein the mesenchymal stem cells are associated in a three-dimensional format, and wherein said chemically-defined serum-free medium comprises (1) a chemically defined minimum essential medium; (2) ascorbate or an analog thereof; (3) an iron source; (4) insulin or an insulin-like growth factor; (5) at least one chondroinductive agent or factor, wherein said at least one chondroinductive agent or factor is TGF-β3; and (6) a simple sugar, said simple sugar being present in an amount of from about 3g/l to about 7g/l.

91. The process of Claim 90 wherein said mesenchymal stem cells are isolated, culture expanded human mesenchymal stem cells.

92. The process of Claim 90 wherein the mesenchymal stem cells are condensed into close proximity.

93. The process of Claim 92 wherein the mesenchymal stem cells are present as packed cells or a centrifugal cell pellet.

94. The process of Claim 90 wherein said TGF-β3 is present in said medium in an amount of at least 5 ng/ml.

95. The process of Claim 94 wherein said TGF-β3 is present in said medium in an amount of from 5 ng/ml to 15 ng/ml.

96. The method of Claim 90, and further comprising placing said cells in a rigid porous vessel.

97. The method of Claim 96 wherein said rigid porous vessel is a ceramic cube.

98. The method of Claim 90 wherein said simple sugar is glucose.

99. The method of Claim 98 wherein said glucose is present in said serum-free medium in an amount of about 4.5 grams per liter.

EVIDENCE APPENDIX

No Declarations pursuant to 37 CFR 1.130, 37 CFR 1.131, or 37 CFR 1.132 were submitted by Applicants during prosecution of this Application.

RELATED PROCEEDINGS

There are no related appeals or interferences.